Relationship between structure and function of Helix pomatia alpha-hemocyanin

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Publication date:
1974

CHAPTER 7

SUMMARY AND CONCLUSIONS

Hemocyanin is an oxygen binding protein, present in some invertebrates. The very large α-hemocyanin from the Roman snail, Helix pomatia, which binds 180 oxygen molecules and has a molecular weight of 9×10^6, has been studied. The focus of this work has been on the relationship between the structural and functional properties of this large biological system.

Hemocyanin can be reversibly dissociated into half, one-tenth, and one-twentieth molecules. When different dissociation states coexist in solution their relative amount is independent of the total protein concentration. An explanation for this behaviour is presented: the concept of microheterogeneity.

We have determined the oxygen binding properties of (i) whole molecules, containing only native subunits; (ii) hybrid molecules containing inactivated one-tenth subunits, which were unable to bind oxygen, as well as native one-tenth subunits; and (iii) native protein, dissociated into one-tenth subunits. These experiments were carried out at moderate ionic strength (0.1), at pH 8.2. They indicate that interactions between native one-tenth subunits are essential for cooperative oxygen binding. It is concluded that the cooperative oxygen binding unit is larger than a one-tenth subunit.

More evidence for the linkage between cooperative oxygen binding and changes in the intersubunit interactions was obtained at high ionic strength (1.1), at pH 8.2. Under these conditions oxygen binding is cooperative. The ligand free protein is largely present as half molecules. Binding of oxygen results in an abrupt, complete dissociation of the half molecules into one-tenth subunits. Evidence is presented that under the prevailing conditions the observed cooperativety is almost completely due to ligand linked dissociation. These oxygen binding properties have been compared to those under more physiological conditions. It is suggested that formation of a closed ring of five one-tenth subunits (i.e., association to a half molecule) is a necessary condition to constrain the protein to a state with low oxygen affinity, which in turn is a prerequisite for cooperative oxygen binding.

The results summarized above are based mainly on measurements of oxygen affinities of various dissociation states. We have also tried to obtain information about structural changes in the undissociated, native protein, which binds oxygen cooperatively. It has been found that hemocyanin can be fixed in a state with low oxygen affinity by crosslinking with a bifunctional reagent (dimethylsuberimidoester) under ligand free conditions. Similarly, crosslinking of the liganded protein results in a derivative with high oxygen affinity. The results show that liganded and ligand free hemocyanin have different structures. The ability to fix hemocyanin in different states may facilitate further studies of the structural changes that underlie the process of cooperative oxygen binding.

The final part of this thesis reports an investigation of the kinetics of the reaction of hemocyanin with oxygen. Stopped flow experiments show that the combination of deoxy hemocyanin with oxygen, as well as the dissociation of oxy hemocyanin are autocatalytic. Both the combination and dissociation rate constants contribute to the cooperativety of oxygen binding. Temperature jump relaxation experiments have been carried out at oxygen saturations between 0.7 and 1.0. In this range oxygen binding can be described by one set of kinetic constants. This strongly suggests that no structural transitions take place at oxygen saturations above 0.7.

The most salient conclusions concerning the structure - function relationship of Helix pomatia α-hemocyanin are:

1. oxygen saturated and ligand free protein have distinctly different structures, which are characterized by a high and a low affinity for oxygen, respectively;
2. both the low and the high affinity states seem to be stabilized over rather large ranges of oxygen saturation; at present, no evidence for intermediate states has been obtained;
3. only native, undissociated protein (whole and half molecules) can occur in a state with low oxygen affinity; this suggests that only the associated system (a closed ring of one-tenth subunits, _i.e._, a half or whole molecule) is able to undergo structural transitions, which result in cooperative oxygen binding;
4. a strong linkage is observed between oxygen binding and subunit interactions;
5. both the combination and dissociation rate constants contribute to the cooperativety of oxygen binding.